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RESEARCH ARTICLE

Ecotoxicological efficiency of advanced ozonation processes with TiO_2 and black light used in the degradation of carbamazepine

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Abstract The aim of the present study was to evaluate the ecotoxicological efficiency of two advanced ozonation processes (AOzPs), the catalytic ozonation (O_3/TiO_2) and the photocatalytic ozonation (O_3/TiO_2/black light), in the remotion of carbamazepine. The ecotoxicological efficiency was assessed through the use of lethal and sublethal assays with species *Vibrio fischeri* and *Daphnia magna*. Results demonstrated that the AOzPs presented an efficiency of carbamazepine removal higher than 99% (carbamazepine < 2 μg/L) after 12 min of treatment. Relatively to ecotoxicological evaluation, application of acute assay to *V. fischeri* and chronic assay to *D. magna* allowed us to highlight that these technologies may form some transformation products that induce toxicity in the bacteria and the crustacean, once these organisms exposed to the undiluted solutions (100%) showed a decrease in the bioluminescence (vibrio) and end up dying before and during the first reproduction (daphnia). Despite that, when the chronic results obtained with the diluted solutions (50 and 25%; important to assess a more realistic scenario considering the dilution factor at the environment) were analyzed, no mortality at the mothers was observed. Compared to a carbamazepine solution (200 μg/L), diluted solutions improved of the reproduction parameters, and no toxic effects in the juvenoid system and in the embryonic development were observed. Relatively to the ecdysteroid effect of a carbamazepine solution (200 μg/L), only the photocatalytic ozonation treatment was able to remove the action of the drug. These results highlight the importance of complementing chemical analysis with ecotoxicological bioassays to assess the best technology to improve the surface water and effluent quality.

Keywords Advanced ozonation processes · Carbamazepine · Toxicity · *Daphnia magna* · *Vibrio fischeri*

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Introduction

Pharmaceutical products (PPs) are currently not considered under water quality regulations. However, studies about their potential effect on the environment have become more common in recent years, leading to the inclusion of these chemicals among the so-called emerging pollutants (La Farre et al. 2008; Stuart et al. 2012; Li et al. 2015). PPs can be introduced into aquatic systems by industrial wastes, veterinary uses, or domestic and hospital wastewaters (Kasprzyk-Hordern et al. 2008; Monteiro and Boxall 2010; Sauvé et al. 2012). Recent data show that PPs are quite refractory to conventional operations in wastewater treatment plants (WWTPs), being reportedly detected in effluents, surface water and groundwaters, and even drinking waters (Mompelat et al. 2009). PPs are evidenced or suspected to

promote toxic effects on living organisms even when present at concentrations as low as nanograms per liter (Focazio et al. 2008; Dietrich et al. 2010; Sousa et al. 2012; Galus et al. 2013; Almeida et al. 2014; Galus et al. 2014; Candido et al. 2016; Oropesa et al. 2016). Hence, in this scenario, developing and testing technologies that may be integrated into WWTPs in an easier and economically sustainable way, for a better response in the removal of PP emissions to the aquatic environment, are necessary. Several authors agree that among the approaches that have been studied for the removal of these pollutants, advanced oxidation processes (AOxPs), that rely on free radicals, mainly hydroxyl radicals, are a suitable option (Cesaro and Belgiorno 2016; Chavez et al. 2016). Their application has been studied for the degradation of several kinds of emerging contaminants, including PPs (Beltran et al. 2008, 2009a; Rodriguez et al. 2012; Sousa et al. 2012; Espejo et al. 2014a, b; Miralles-Cuevas et al. 2014; Barbosa et al. 2016; Tokumura et al. 2016; Wang and Wang 2016; Gomes et al. 2017; Hassania et al. 2017; Yang et al. 2017), personal care products (Kim et al. 2009; Li et al. 2012; Sousa et al. 2012; Wang and Wang 2016; Gomes et al. 2017; Yang et al. 2017), pesticides (Beltran et al. 1999; Rivas et al. 2001; Rodríguez et al. 2004; Jović et al. 2013; Rozas et al. 2016), and sex hormones (Suri et al. 2007; Rodriguez et al. 2012), and dyes (Grčić et al. 2014). In this context, several AOxPs have been used, with different results concerning the efficiency rates and final water quality. Among AOxPs, ozone processes are of great interest because of the capability of ozone to react and produce hydroxyl radicals alone or in combination with other agents, such as peroxides, catalysts, and/or radiation (Beltran 2004). Several studies have concluded that these ozone processes, also named advanced ozonation processes (AOzPs), are effective technologies to remove PPs from water (Ternes et al. 2003; Huber et al. 2003; Ikehata et al. 2006; Ibañez et al. 2013; Beltran and Rey 2017; Gomes et al. 2017). The efficiency of these processes depends on the compound to be removed, as well as on operating conditions (ozone concentration, pH, time, type of water matrix, etc.) (Cokgor et al. 2004; Cesaro and Belgiorno 2016). On the other hand, ozonation can produce by-products with pharmacological activity that can even be more toxic than the parent compounds for aquatic organisms (Quero-Pastor et al. 2014). According to Huber et al. (2005), in a single ozonation process, a low level of PP mineralization is usually achieved with the ozone doses typically applied in water treatments. If ozonation is combined with other agents, such as radiation or/and catalyst, i.e., titanium dioxide (TiO_2), another AOzP is constituted and more hydroxyl radicals are generated. These radicals react unselectively with organic compounds and ozonation by-products to eventually reach water mineralization to CO_2 and H_2O (Beltran 2004).

Carbamazepine (CBZ) is a very used psychiatric drug that is slowly degraded by sunlight, and as a consequence, it is one of the most detected PPs in freshwater ecosystems worldwide (Calisto et al. 2011; Bahlmann et al. 2012; Almeida et al. 2014). In this context, CBZ represents an interesting indicator of anthropogenic contamination due to their widespread detection in the environment and their potential relationship with water contamination levels, giving an indication of the presence of other chemicals related to human activities (Ruzicka et al. 2011). For instance, CBZ has been determined at a maximum concentration of 68 μg/L in Jarama River (Madrid) (Valcarcel et al. 2011). It is a pseudo-persistent compound because of its physico-chemical properties and its continual discharge into aquatic media via WWTP effluents which means a chronic exposure for the aquatic organisms. Relatively to its ecotoxicological effects, CBZ causes sublethal effects in several biological processes such as development, growth (Cleuvers 2003; Jos et al. 2003; Crane et al. 2006; Dietrich et al. 2010; Zhang et al. 2012; Lamichhane et al. 2013), and reproduction (Dietrich et al. 2010; Lamichhane et al. 2013; Galus et al. 2013, 2014) in aquatic organisms. Concerning to the crustacean *D. magna*, a previous study reported that concentrations of CBZ, between 10 and 200 μg/L, act on its reproduction namely (i) decreasing the number of juveniles per female, (ii) inducing abnormalities and sex alterations in embryos exposed to this compound through the mother, and (iii) acting as weak juvenile hormone analog (Oropesa et al. 2016).

It should be highlighted that WWTPs supplied with preliminary clarification followed by an aerator tank with activated sludges (Ternes 1998; Zhang et al. 2008; Gimeno et al. 2016) or an anaerobic/anoxic/aerobic process combined with membrane bioreactor (Xue et al. 2010) do not effectively remove CBZ. Then, new more effective AOxPs should be considered for its complete removal from WWTP effluents. The precautionary principle suggests preventing its release to the aquatic environment by limiting its presence in the main source of emission, i.e., principally in WWTP effluents. For this reason, some researchers have applied treatments of ozonation (Ternes et al. 2002; McDowell et al. 2005), as well as different AOxPs (including AOzPs) such as UV/H_2O_2-induced photolytic degradation (Vogna et al. 2004; Rozas et al. 2016), UV/chlorine (Zhou et al. 2016), photocatalytic degradation with TiO_2 (Doll and Frimmel 2005; Martinez et al. 2011; Jelic et al. 2013; Czech and Buda 2015), direct photolysis (Chiron et al. 2006; Calza et al. 2012), aerobic biological oxidation followed with photocatalytic ozonation with UVA black light and Fe(III) or Fe_3O_4 as catalysts (Espejo et al. 2014a, 2014b; Gimeno et al. 2016), and generation of sulfate radicals ($\cdot SO_4^-$) from UV/persulfate and UV/peroxymonosulfate (Deng et al. 2013) to improve CBZ removal rate. Concentrations of the drug as well as ecotoxicological parameters need to be determined in the treated solutions (solutions after application of AOxPs). Therefore, the evaluation of both the removal rate of the compounds and the lack of ecotoxicity in the treated solutions of the chemical process applied is neccesary.

Thus, taking into consideration the results obtained in the previous study with CBZ (Oropesa et al. 2016), the main aims

of the present research were to (i) evaluate the removal efficiency of CBZ solutions (200 μg/L) by two technologies of AOzPs: (a) catalytic ozonation (O_3/TiO_2; T1) and (b) photocatalytic ozonation (O_3/TiO_2/black light; T2) and (ii) ecotoxicological assess of the treated solutions through acute and chronic assays. The ecotoxicological assessment was performed with biological models belonging to different trophic levels, the bacteria *Vibrio fischeri* (decomposer) and the microcrustacean *Daphnia magna* (first consumer) assessing lethal and sublethal endpoints (reproductive and endocrine parameters). To the best of our knowledge, this is the first study of AOzP ecotoxicological removal efficiency that integrates lethal and life cycle endpoints. Despite the lethal bioassays being very easy to perform and relatively sensitive, which make them the most used by the researchers to assess the efficiency of different water treatment systems, the sublethal effects in the biota are more likely to occur due to the continuous release of low concentrations of PPs. Thus, sublethal effects would be expected for environmentally relevant concentrations of PPs (ng/L–μg/L), and these may not be detected by acute toxicity tests, which justify the use of life cycle endpoints of *D. magna* in the present study. Furthermore, the possible presence of by-products (resulting from these chemical technologies) makes more relevant the use of bioassays that cover most of the life cycle of the species (chronic tests). This is because oxidation by-products can change some physiological parameters that at medium and long term may compromise the reproduction and growth of this species even though they may have a low toxicity (revealed through acute tests). These effects may be masked in short-term assays, but are highlighted at chronic tests. Apart from the environmental relevance of the chronic test with *D. magna*, it is important to highlight its complexity, the time-consuming as well as the need to have an appropriate infrastructure.

Material and methods

Chemicals and experimental procedures

Carbamazepine ($C_{16}H_{12}N_2O$; CAS No. 298-46-4; ≥ 98% purity) was provided from Sigma-Aldrich Quimica S.A., while hydrogen peroxide was obtained from Merck. Catalyst AEROXIDE® TiO_2-P25 (size of particle 30–50 nm; 99.9% purity) was purchased from Degussa (Germany). According to Ohtani et al. (2010), P25 is a simple mixture of anatase (78–85%), rutile (14–17%), and amorphous (0–13%) titania with different ratios depending on the sample production batch and position in the package with anatase/rutile ratios varying from 4.9 to 5.7. TiO_2 P25 is the most used catalyst in photocatalytic oxidation of organics in water since it is very stable and active. For instance, Aguinaco et al. (2012) showed these properties while studying the photocatalytic ozonation of diclofenac after four consecutive runs of 2-h duration with the same TiO_2 catalyst.

All experiments were carried out in a 4-L borosilicate cylindrical reactor equipped with bubbling through a porous plate situated at the reactor bottom, mechanical agitation, and devices for measuring temperature and pH. The reactor was situated in the center of a 50 × 30 × 30 cm black wooden box where 4 15 W black light lamps (Lamp 15 TBL HQ Power TM Velleman®) were placed in the corners inside the box. These lamps emit radiation in the range of 350–400 nm centered at ~ 370 nm. Schematic view of the experimental setup is available in Espejo et al. (2014a). Parker reagent (Hatchard and Parker 1956) was used as actinometer to determine the incident photon flux, I_0, in the photoreactor, that was found to be 1.4×10^{-6} Einstein/s.

For photocatalytic ozonation, the experimental procedure was started by turning on the lamps and waiting 30 min to reach their emission stationary state. Meanwhile, the reactor, outside the wooden box, was charged with an aqueous solution (Milli-Q purified water, Millipore) containing CBZ at a nominal concentration of 200 μg/L. At this concentration, although slightly higher than the highest concentration found in surface water (68 μg/L; Valcarcel et al. 2011), an accurate and fast quantitative chemical analysis with available equipment can be accomplished. However, changes of TOC or COD of the aqueous solutions could not be followed because of the very low mass content of the treated water that makes inaccurate the measurements with the available equipments. Also, an amount of TiO_2 was added to the aqueous solution so that catalyst concentration was 250 mg/L (Beltran 2004; Quiñones et al. 2015). Regardless of the presence of radiation, the abovementioned 30 min were also intended for reaching CBZ equilibrium adsorption. In any case, however, CBZ adsorption on TiO_2 was negligible (less than 3% after 2-h treatment).

An ozone-oxygen gas mixture was continuously fed through the porous plate with a flow rate of 35 L/h and an ozone concentration of 13 mg/L (Beltran 2004; Mena et al. 2012; Quiñones et al. 2015). In all runs, at regular intervals, samples were withdrawn from the reactor and vacuum pressure filtered through 0.45-μm Sartorius membranes. Experiments were carried out in triplicate. Although, in all AOzPs, CBZ was reduced below the detection limit (2 μg/L) in less than 12 min, the reaction time was prolonged for 60 min to check the ecotoxicity of product intermediates.

The treated solutions with a pH of 5 were neutralized with 1 N NaOH from Panreac Química S.A. prior to the beginning of the ecotoxicity tests.

Chemical analysis

Analysis of CBZ concentration was conducted by high-performance liquid chromatography (HPLC) using a

LaChrom Elite equipment (VWR International-Hitachi, Barcelona, Spain) provided with a Millipore Iberica S.A.U. (Madrid, Spain) Synergi Hydro-RP column (250 × 4.60 mm, particle size 4 μm, 80 Å), a L-2450DA detector, and an EZ Chrom software (VWR International) for data treatment. The injection volume was 99 μL. The mobile phase consisted of a binary mixture of solvents: acetonitrile (A) and acidified water with 0.1% formic acid (B). A 40 min linear gradient from 10 to 100% of A with a flow rate of 0.2 mL/min mobile phase gradient program was used. The separation was monitored at absorbance wavelength and retention time of 211 nm and 27.2 min, respectively. Detection limit for accurate measurements of concentrations was 2 μg/L. Analysis of standard solutions was repeated in triplicate to establish the precision of the method that resulted to be ± 2.0%, while accuracy was 1.3%. Also, concentrations of dissolved ozone and outlet ozone gas concentrations were followed with the Indigo method (Bader and Hoigné 1981) and with an Anseros Ozomat ozone gas analyzer, respectively. After 60 min of reaction, concentration of dissolved ozone reached at stationary value of 2.65 and 0.36 mg/L, in catalytic and photocatalytic ozonations, respectively, while the ozone gas concentration at the reactor outlet was 12.8 and 11.2 mg/L for both ozonation methods. Hydrogen peroxide concentration was determined through the cobalt-bicarbonate method (Masschelein et al. 1977).

Ecotoxicological assessment

Light inhibition bioassay of V. fischeri

The bacteria *V. fischeri* was provided by Dr. Lange GmbH & Co KG, Düsseldorf like a Toxkit. Luminotox® was used to evaluate the inhibition luminescence of the marine bacteria *V. fischeri* (NRRL B-11177), according to the "Dr. Lange luminescent bacteria test" protocol following ISO 11348-2 (1998). The bacteria *V. fischeri* was supplied as liquid-dried solution. This solution was stored at − 20 °C and rehydrated prior to the test. Tests were carried out on 100.0% (undiluted solution, i.e., solution resulting from AOzPs), 50.0, 25.0, 12.5, and 6.25% (*v*/*v*; dilutions, with 2% NaCl, resulting from AOzP processes). Also, a solution of 200 μg/L of CBZ (in 2% NaCl) was tested as positive control. Two replicates, per treatment, were used. The inhibition of the bacteria natural light emission was measured against a non-toxic control (2% NaCl solution). The samples were maintained at temperature of 15 ± 0.5 °C. The pH of treated solutions was in the range 6.5–7.0. For each sample, bioluminescence was measured before and after the desired contact period (15 and 30 min). The inhibiting effect was expressed as percentage of bioluminescence inhibition (*H*, %) and estimated using the following equation:

Inhibiting effect (*H*) = ((corrected I_0 − I_{30}) / corrected I_0) × 100

$$H(\%) = (\mathrm{IT_t}/(\mathrm{IT_0} \times \mathrm{fc}))$$
$$\mathrm{fc} = \mathrm{ICt}/\mathrm{IC_0}$$

where fc is the correction factor based on the control (taking into account the natural decrease in luminous intensity), IC_0 and IT_0 are the initial luminescence intensities of control and test samples, and ICt and ITt are the luminescence intensities of the control and test samples after "*t*" minutes of exposure time (15 and 30 min).

D. magna bioassays

For developing the lethal and reproduction ecotoxicity bioassays, *D. magna* was obtained from continuous cultures maintained in the lab at 20 ± 1 °C with ASTM hard water (ASTM 1998) and a 16-h light/8-h dark photoperiod at a light intensity of 100–1000 lx. Animal density was 15 animals per 800 mL of ASTM medium. Three times per week, Daphnids were fed with algae (*Pseudokirchneriella subcapitata*) with a density of 3.0×10^5 cells/mL daphnia (an equivalent carbon content of 2.65 mg C/mL) and enriched with a standard organic extract from the algae *Ascophyllum nodosum* (Baird et al. 1989), at a concentration of 4.0 mL/L. Under these conditions, partenogenic reproduction occurs and practically all the offspring produced are female (> 98%). The sensitivity of the daphnids was checked monthly with potassium dichromate ($K_2Cr_2O_7$) from Merck, and an EC_{50} (24 h) of 1.85 mg/L was obtained, which is within the range of the 0.6–2.1 mg/L stipulated in the ISO 6341 (1996) to ensure test validity.

Contrary to *V. fisheri* bioassay, the concentration of 200 μg/L of CBZ was not integrated into the *D. magna* bioassays, since the evaluation of its lethal and chronic toxic effects had already been reported in a previous work (Oropesa et al. 2016).

Mortality/immobility bioassay

The acute bioassay was performed according the Guideline for Testing of Chemicals No. 202: *D. magna* acute immobilization test (OECD 2004). Twenty juveniles (< 24 h) obtained between the third and fifth brood, four replicates, and five organism per replicate were used for each test solution. Acute ecotoxicity of the treated solutions to *D. magna* was determined after 48 h of exposure using the following solutions: 100.0% (undiluted solution, i.e., solution resulting from AOzPs), 75.0, 50.0, 25.0, and 12.5% (*v*/*v*; dilutions, in ASTM medium, resulting from AOzP processes).

Chronic reproduction bioassay

To evaluate the chronic toxicity of the treated solutions to daphnids, a standard 21-day bioassay was carried out following the Guideline for Testing of Chemicals No. 211: *D. magna* reproduction test (OECD 2012). Daphnids (less than 24 h old), randomly selected among those produced in the third and fifth broods from a single clone females, were individually exposed in 100-mL glass beakers containing 50 mL of the test medium. These organisms were exposed to negative control (ASTM hard water) and to the following solutions: 100.0% (undiluted solution, i.e., solution resulting from AOzPs), 50.0, and 25.0% (*v*/*v*; dilutions, in ASTM medium, resulting from AOzP processes), 10 replicates per concentration each containing one daphnid. Test solutions were renewed three times a week, and at the same time, the organism were fed with *P. subcapitata* (3.0×10^5 cells/mL/daphnia) and Marinure extract®. These maintenance procedures were similar to those for the stock cultures. Dissolved oxygen, temperature, conductivity, and pH were measured once a week. The endpoints assessed were daphnids' mortality, growth, molting inhibition, age at the first reproduction, total number of broods produced, total number of viable offspring produced per female, embryo toxicity (number of arrested eggs, number of abnormalities in antennules or tails of offspring), and male offspring production. Growth was determined by measuring the body length from the top of the head to the base of the tail spine of the adults. The length and the morphology of the first antenna were used as the basis for sex determination according to GTC: No. 211 (OECD 2012). Body length of adults, sex, and abnormality development of offspring were observed and counted using an stereomicroscope (Olympus SZX9, Olympus Optical Co., Ltd., Tokyo, Japan) coupled to an image analysis software JENOPTIK Optical Systems (Version 2.8.8).

Statistical analysis

Data from chronic test in *D. magna* were checked for assumptions of normality and variance homogeneity using the Kolmogorov-Smirnov and Levene's tests, respectively. As the ANOVA assumptions were not met, the non-parametric Kruskal-Wallis test was used. The Dunn's multiple comparison test was subsequently applied for pairwise comparisons of each treatment with the control group by selecting p values of 0.05, 0.01, and 0.001 as the significant levels. All statistical analyses were carried out using the Statistic software SPSS v. 21.

Results and discussion

Efficiency of applied AOzPs in removing CBZ

Two schemes of AOzPs (T1: O_3/TiO_2 and T2: O_3/TiO_2/black light) were delineated to assess the removal efficiency of CBZ. At the end of both AOzP processes (T1 and T2) (60 min), the concentration of CBZ was below the detection limit (< 2 μg/L), which highlights the removal efficiency of this drug by the advanced ozonation technologies applied.

Studies performed with the UV-A irradiation or ozone-free photocatalytic oxidation ($UVA/TiO_2/O_2$) of CBZ (10 mg/L) revealed lower removal rate (75% after 120 min or 50% after 45 min, respectively) (Jelic et al. 2013; Czech and Buda 2015) than the CBZ removal with the O_3/TiO_2 and O_3/TiO_2/black light processes (starting with 200 μg/L CBZ, which is a more realistic concentration) (> 99% removal after 12 min) applied in our study. CBZ at the conditions applied here in catalytic and photocatalytic ozonations was completely removed in less than 12 min. In fact, 10 min were needed in photocatalytic ozonation to reduce the concentration of CBZ under the quantification limit, while 12 min were necessary in catalytic ozonation. Also, for the total removal of CBZ, ozone consumption was 126 and 81 mg per mg CBZ removed for catalytic and photocatalytic ozonation, respectively. This means that removal of CBZ requires less ozone in photocatalytic ozonation likely due to the higher formation of hydroxyl radical in the photocatalytic system. On the other hand, the ozone transfer percentage defined as ozone consumed per total ozone fed during 60 min was 14.7 and 16.2% for catalytic and photocatalytic ozonations, respectively. However, nothing can be said about the reactivity of transformation products (TPs) formed in these processes since TOC could not be determined. According to literature, the photocatalytic process always requires less consumption of ozone per milligram TOC removed (Aguinaco et al. 2012). In this study, however, the small starting concentration of CBZ likely leads to the slight differences noticed between both processes.

According to several authors, the photodegradation of CBZ generates TPs such as aldehydes, ketones, hydroxyl derivatives, and acridine (Chiron et al. 2006; Kosjek et al. 2009; Martinez et al. 2011). After CBZ $UVA/TiO_2/O_2$ photocatalytic degradation, Jelic et al. (2013) found the monohydroxy derivatives 2-hydroxy-CBZ and 3-hydroxy-CBZ and the isomeric forms of TP 223 as main TPs. A previous study by Doll and Frimmel (2005) also detected CBZ hydroxyl derivatives (10,11-dihydro-CBZ-10,11-epoxide, hydroxy-CBZ, dihydroxy-CBZ) and acridine after O_2/TiO_2 photocatalysis treatment. Regarding the ozonation treatment, CBZ spiked to natural water at 236 μg/L was completely oxidized by ozone (dose 1 mg/L) resulting in 1-(2-benzaldehyde)-4-hydro-(1H,3H)-quinazoline-2-one (BQM) and 1-(2-benzaldehyde)-(1H,3H)-quinazoline-2,4-dione (BQD) (McDowell et al. 2005). Also, Hubner et al. (2014) have identified BQM and some other 12 TPs during CBZ ozonation conducted in a secondary effluent of a WWTP. On the other hand, as far as we know, literature does not show any work where TPs of CBZ catalytic or photocatalytic ozonation have been detected or identified.

In spite of the lack of data regarding TP formation, some comments can be said about possible differences between TiO_2

catalytic ozonation and TiO_2-black light photocatalytic ozonation. These differences could likely be due to the presence of a radiation source of less than 387-nm wavelength. During TiO_2 catalytic ozonation, formation of TPs is due to both direct reactions of organics present with ozone (Mvula and von Sonntag 2003; Leitzke and von Sonntag 2009; Beltran et al. 2009b, 2010) and hydroxyl free radicals formed from the decomposition of adsorbed ozone (Agustina et al. 2005). However, during TiO_2-black light photocatalytic ozonation, in addition to these reactions, other pathways promoted by the radiation source play an important role. Thus, radiation excites electrons of the valence band of TiO_2 to jump to the conduction band. In this way, a couple of charge carriers are formed: very oxidizing centers called holes at the valence band and electrons at the conduction band. Holes at the valence band may directly oxidize adsorbed pollutant molecules or react with adsorbed water to yield adsorbed hydroxyl radicals (Zhang et al. 2003; Kamble et al. 2007; Sanchez et al. 2011). Also, electrons at the conduction bands are trapped by ozone (and also by oxygen used to generate ozone) to form more hydroxyl radicals and even hydrogen peroxide which triggers more free radical reactions (Staehelin and Hoigné 1982). It should be noted that addition of ozone to a photocatalytic oxidation system also presents beneficial results since it reduces or stops the electron-hole recombination reaction that inhibits the oxidation process (Mena et al. 2012). Accordingly, similar and different TPs are expected to be formed from these two oxydizing ozone processes.

In any case, AOxPs (including AOzPs) may offer additional treatment value to CBZ removal (Keen et al. 2012), though complete oxidation and mineralization are not always achievable and stable TPs formed during AOzPs may also pose environmental risks (Agüera et al. 2005). The ecotoxicity of the treated solutions with TPs formed during treatment and environmental transport is of major importance when assessing the suitability of water treatment options and in determining environmental risks associated with active pharmaceutical ingredients in the environment.

The chemical analysis of the solutions obtained after application of AOxPs to CBZ is not reliable enough to determine the most efficient treatment method because CBZ TPs can be generated and these substances can exercise adverse biological effects in aquatic organisms. Hence, the importance of assessing the ecotoxicity of the final solutions is justified to (i) guarantee that these treated solutions can be safely released to the environment and (ii) complement the chemical evaluation in order to determine the real efficiency of the applied process.

Ecotoxicological assessment

Acute bioassays

Bacterial organism bioluminescence is correlated with the intrinsic metabolic process for obtaining energy. Thus, the toxicity observed is due to the interference of chemicals with the metabolism of the bacterial. Therefore, the light output of the luminescent bacteria exposed to CBZ and treated solutions and their dilutions was compared with the light output of a blank control (NaCl, 2%).

The exposure to undiluted solutions, from T1 and T2 processes, induced a significant bioluminescence decrease of 32 and 25%, respectively (Fig. 1). This effect is probably due to the presence of TPs resulting from the technologies applied, once our results also showed that the concentration of 200 μg/L of CBZ did not induce toxicity in the bacterial *V. fischeri* ($H = -2.09\%$). This hypothesis is also supported by the results reported by Jos et al. (2003) who estimated an EC_{50} (CBZ), after 15 and 60 min of exposure, of 78 and 64 mg/L, respectively. Moreover, the toxic effects of AOzP treatments to the bacteria induced were also observed by He et al. (2016) who reported a decrease of 20% of bioluminescence of the *V. fischeri*, after 45 h of the oxidation process with an immobilized TiO_2 photocatalysis under simulated solar irradiation. In contrast, Czech and Buda (2015) using the photocatalytic process with nanocomposites of TiO_2/SiO_2 (size of particle 20–30 nm) to degrade CBZ reported that the solutions obtained showed lower toxicity for *V. fischeri* than the initials. From results obtained for all tested solutions, it was not possible to estimate the value of EC_{50}.

Regarding the results obtained with the crustacean *D. magna*, after 48 h of exposure, the application of the selected AOzPs seems to be efficient to remove the acute toxicity of CBZ, once the results indicated an immobilization of 10% after photocatalytic ozonation (TiO_2 and black light; T2) and no toxicity after the treatment with catalytic ozonation (TiO_2; T1). The slight effect observed at T2 is equivalent to the NOEC (no observed effect concentration); therefore, this treatment did not show acute toxicity to *D. magna*. Our results are in line with those of Li et al. (2011) who showed that a resulting solution from 20-min CBZ ozonation (50 mg/L) was not acutely toxic to *D. magna*. On the contrary, Rizzo et al. (2009) and Jelic et al. (2013) reported 20 and 20–30% *D. magna* immobilization after 48 h of exposure to solutions of CBZ (5 and 10 mg/L) under TiO_2 photocatalytic oxidation with black light process, for 60 and 30–120 min, respectively. The acute effects observed in these latter studies were higher than the current ones, probably due to some different conditions at the treatment processes, such as the higher amount of TiO_2 used (0.4 and 0.8 g/L).

Chronic bioassay with D. magna

The ecotoxicological assessment through sublethal endpoints revealed some effects induced by AOzPs, which were not detected in acute bioassays. The increment of the time of exposure (> 48 h; performed at acute test) induced mortality before and during the first reproduction in the daphnids

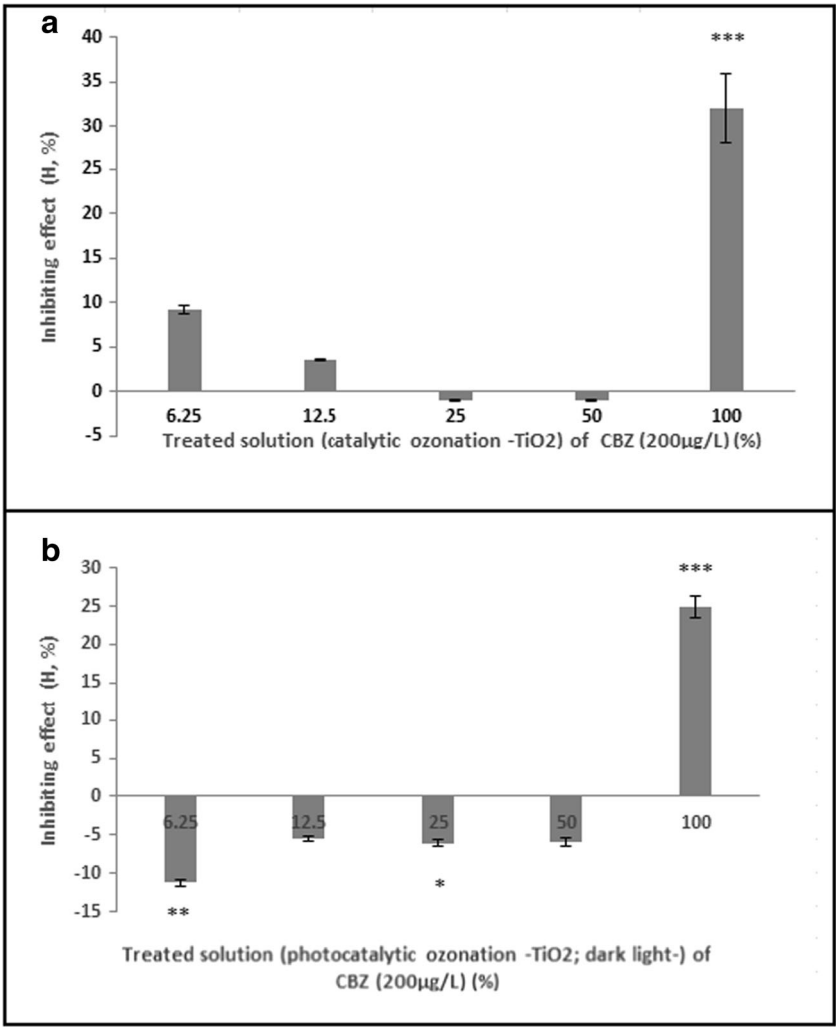

Fig. 1 Inhibiting effect (H, %) on the luminiscence of bacteria *V. fischeri* after exposure to solutions of CBZ (200 μg/L) under catalytic ozonation (TiO_2) and photocatalytic ozonation (TiO_2; dark light) [**a**, **b**, respectively] (100%) with their corresponding dilutions (50, 25, 12.5, and 6.25%). Values are the mean with its corresponding standard error bars which represent the SEM. *, **, *** indicates significant differences from the control group mean (Dunn's multiple comparison test: $p \leq 0.05$; $p \leq 0.01$; and $p \leq 0.001$, respectively)

exposed to the 100% of the treated solutions (T1, T2). Hence, the daphnids exposed to 100% of T1 reach one reproduction (survive during 10–12 days), and the daphnids exposed to 100% of T2 died 4 days after the start of the test. These effects probably were correlated with some intermediate compounds produced at the treatment processes. One of the principal by-products of AOzPs is the hydrogen peroxide; however, at the present study, we think that the toxicity observed in the 100% treated solutions is not correlated with the levels of hydrogen peroxide present in the final solutions (0.54 mg/L), but with the formation of CBZ TPs in the treatment processes. This suggestion takes into consideration results from a *D. magna* chronic study with hydrogen peroxide (0.32, 0.63, 1.25, 2.5, and 5.0 mg/L) developed by Meinertz et al. (2008), which indicated that concentrations lower than 0.63 mg/L did not induce mortality to the crustacean. Probably other TPs of CBZ oxidation processes, such as isomers of hydroxy and keto derivatives (identified at different photocatalytic processes and formed as a result of oxidative transformation reactions involving non-selective hydroxyl radicals; Jelic et al. 2013), may be responsible for the observed effects, hypothesis that must be tested in future works.

On the other hand, we evaluated the effects in the diluted solutions of the treatments proposed (T1 and T2: 50 and 25%), since it is important to have knowledge of what can really occur at the ecosystem. This is due to the fact that the concentrations of TPs in the environment are much lower than the levels detected at the treated solutions, so the ecotoxicological analysis of the dilution solutions may answer to this subject. Therefore, in relation to reproductive parameters in daphnids exposed to T1 and T2 (50 and 25%), the results showed an age at first reproduction between 10 and 12 days, with statistically significant differences ($p \leq 0.01$) between T2 (50 and 25%) and ASTM (control group) that showed a mean age at first reproduction of 10 days (Fig. 2a). The age at first reproduction of daphnids exposed to T2 was similar to the age in daphnids exposed to 200 μg/L of CBZ (Oropesa et al. 2016). On the other hand, this parameter decreased in daphnids exposed to T1 solutions when comparing with daphnids exposed to 200 μg/L of CBZ. Despite the increase of the number of days

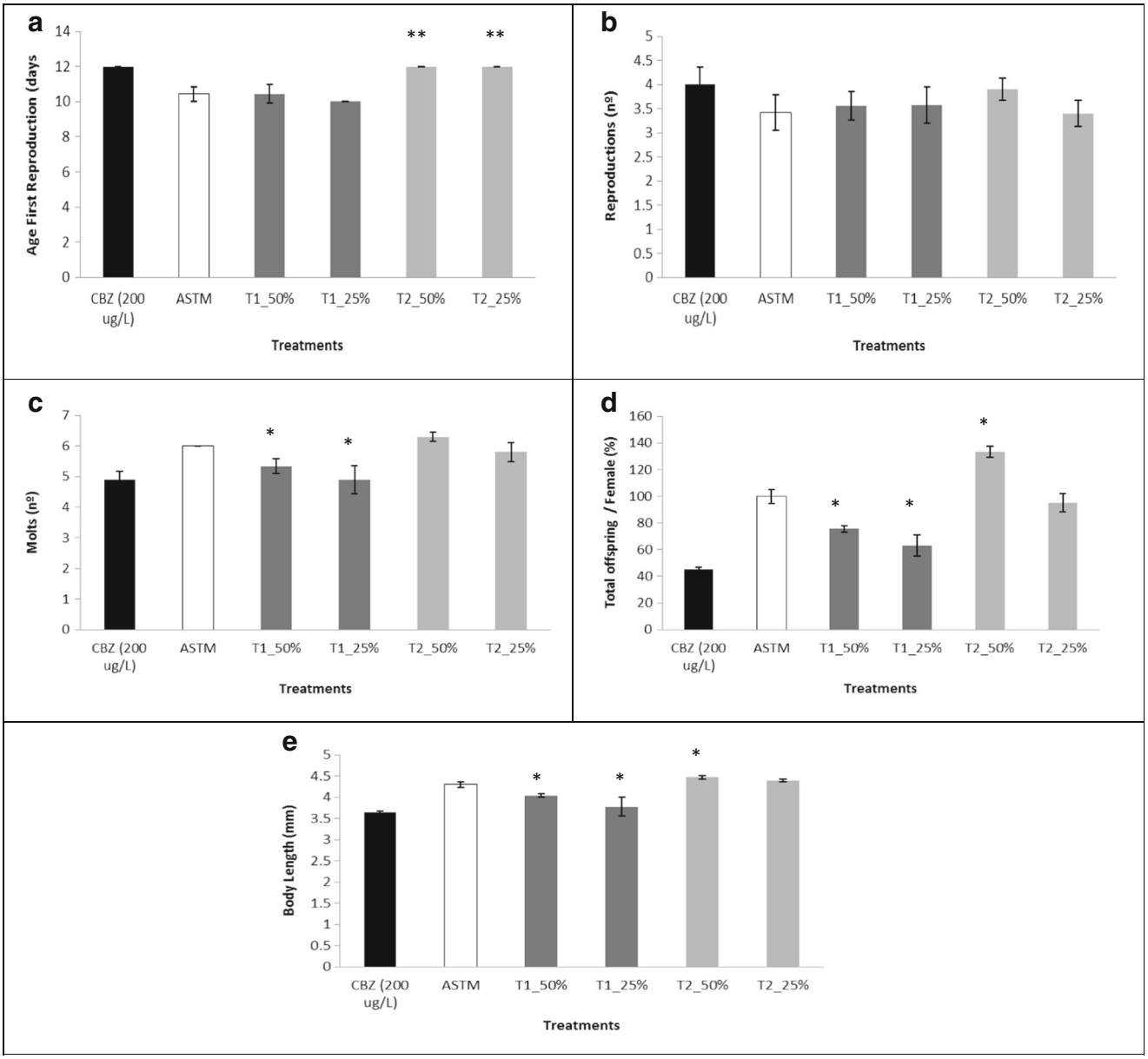

Fig. 2 Age at first reproduction (**a**), number of reproductions (**b**), number of molts (**c**), total number of offspring/female (**d**), body size (**e**) of *D. magna* after the 21 days of exposure to CBZ (200 μg/L) (data obtained from Oropesa et al. (2016)) and treated solutions of CBZ (200 μg/L) [catalytic ozonation (TiO_2)—T1—and photocatalytic ozonation (TiO_2; black light) —T2—with the corresponding dilutions (50 and 25%)]. Values are the mean with its corresponding standard error bars which represent the SEM. *and ** indicate significant differences from the control group mean (ASTM) (Dunn's multiple comparison test: $p \leq 0.05$ and $p \leq 0.01$, respectively)

to the first reproduction observed at the T2 treatment, in general during the 21 days of the tests, the organisms (control and exposed to the diluted treated solutions of T1 and T2) produced, via parthenogenesis, around four progenies of offspring (Fig. 2b). This is in line with the value of this parameter in daphnids exposed to 200 μg/L of CBZ (Oropesa et al. 2016).

Regarding the molting process, only the treated T1 solutions (50 and 25%) induced a statistically significant decrease in the number of molts (four to five molts) comparing to the six molts observed in the control ($p \leq 0.05$), which is similar to that obtained in daphnids exposed to 200 μg/L of CBZ (Oropesa et al. 2016). On the other hand, the frequency of molts obtained with T2 solutions was similar to the control (Fig. 2c).

Taking into account the total number of offspring produced per female over the 21 days, the results showed that T1 treated solutions (50 and 25%) led to a statistically significant decrease of the reproduction rate in comparison with the control group, that produced 52 offspring per female corresponding to 100%, ($p \leq 0.05$) (Fig. 2d). This coincides, as previously indicated, with the reduction in the number of molts per female.

Despite the decrease in the number of offsprings at the T1-treated solutions, with a reproduction rate between 63 and 75%, the treatment was able to improve the reproductive endpoints relatively to the effects observed with 200 μg/L of CBZ, with a reproduction rate around the 45% (Oropesa et al. 2016), or in a study with CBZ and other species of the Daphniidae family, *Ceriodaphnia dubia* that showed a reproduction rate around 55% (Lamichhane et al. 2013). Also, the 50 and 25% dilutions of T2 showed a reproduction rate greater and equal, respectively, than the control. This fact highlights that the increase in the age at first reproduction did not adversely impact the total number of offspring produced per female. The adverse effect yielded by T1 solutions, both in the number of molts per female and in the reproduction rate, may be indicative of disturbance in the ecdysteroid endocrine system of *D. magna* because both physiological processes seem to be regulated by the same hormonal system (Sumiya et al. 2014).

As regards the body length, the T1 treatments (50 and 25%) induced a significant decrease in the species size compared to control, mean body length of 4.3 mm ($p \leq 0.05$), opposite to which was observed for the daphnids exposed to the T2 treatment solutions (50 and 25%) (Fig. 2e). Even though when both treatments (T1 and T2) are compared with previous studies of nominal concentrations of CBZ, we observed the efficiency of the treatment relatively to this parameter, once the body length was always higher than in the daphnids exposed to 200 μg/L of CBZ (Lürling et al. 2006; Lamichhane et al. 2013; Oropesa et al. 2016).

The embryos of the diluted treated solutions had no abnormalities, which is one of the effects induced by CBZ due to its antiecdysteroidal activity (Oropesa et al. 2016). These results highlight the capacity of the treatments to reduce the CBZ concentrations in the solutions and therefore the embryotoxic effect. Thus, with respect to the ecdysteroid activity of CBZ, only the T2 diluted treatment can reverse both effects of the drug on embryo development and on the molting process.

Relatively to the male production, it is important to point that in the control and treated groups, only female offspring was produced, contrary to what has been observed, in a previous study, where 200 μg/L of CBZ produced mixed male/female broods, with a 12% of male offspring (Oropesa et al. 2016). These results are in the line with the previous ones and indicate that at the treated solutions (50 and 25% of T1 and T2), the CBZ was eliminated and their TPs did not act at the endocrine system of the crustacean, contributing to revert the weak juvenoid activity of CBZ.

Consequently, the main adverse effects (decrease in the number of molts, in the total offspring per female and in the body length) observed with T1 (50 and 25% dilutions) must be likely associated with CBZ TPs, a view also shared by Rizzo et al. (2009), who assessed the toxicity to *D. magna* of a solution of CBZ after a TiO_2 photocatalysis treatment. Further, Parkhurst et al. (1981) also observed a decrement in the reproduction rate of *D. magna* after exposure to acridine, one of the TPs generated in T1 treatment (Doll and Frimmel 2005). It is important to point out that the current study was carried out on the basis of the whole effluent toxicity methodology, where the toxic effects of all constituents of an effluent (in our case treated solutions) are assessed to aquatic organisms in order to estimate its potential environmental toxicity. For this reason, in contrast with conventional methods, where the toxicity of single chemicals is controlled, the characterization of intermediate TPs and their possible correlation with their toxicity to the studies species was not one of the aims of this research. Industrial TiO_2 nanoparticles, used as catalysts, ranged from 6.4 to 73.8 nm. These nanoparticles will agglomerate in aqueous solutions (Horst et al. 2010). According to Malato et al. (2009), the size of the aggregates formed by TiO_2 particles in aqueous solutions, at pH = 7, undergoes an increase to 2–4 μm. Hence, the treated solutions were filtered with filters with a pore size of 0.45 μm; therefore, the presence of TiO_2 in final solutions is unlikely. This justifies that the chronic toxicity cannot be attributed to the catalyst presence. On the other hand, the sublethal effects observed on the reproduction of daphnids exposed to diluted treated solutions of T1 cannot be related to the concentration of hydrogen peroxide present in this solution because its concentration was lower than that which causes effects on reproduction parameters of these organisms (Meinertz et al. 2008).

In general, T2 treatment solutions (50 and 25%) incremented the life cycle endpoints tested. A possible explanation to the less toxicity of this treatment to *D. magna* could be due to the additional application of black light that leads to C and N percentage mineralization considerably higher than that from the application of a single TiO_2 photocatalytic treatment, which induces a decrease of the concentration of hydroxyl radicals produced (Marquez et al. 2014).

Overall, the results of the current study show that T1 and T2 treatments (50 and 25%) did not produce any adverse effect in the bioluminescence of *V. fischeri* or in the mobility of *D. magna*. Usually, the ecotoxicity tests used to evaluate the toxicity of the solutions generated after the application of AOzPs are acute tests. If only acute toxicity is assessed, it might be concluded that these solutions do not present risk to the studied species. However, chronic assays may detect sublethal effects, which are missed in short-term tests, as in the case of the chronic test for *D. magna*. Therefore, it could be advisable to carry out not only acute tests but also chronic tests in order to determine the ecological efficiency of treatments applied.

In order to calculate PEClocal (local Predicted Environment Concentration) for the aquatic compartment, the TGD on Risk Assessment (2003) recommends to apply a dilution factor for WWTPs of 10 (1/10). In our case, the resulting solutions were less diluted: 1/2 (50%) and 1/4

(25%). Therefore, under a real environmental scenario (1/10 dilution of the effluent), the treatment proposed (photocatalytic ozonation—TiO_2, black light process—T2) for the remotion of CBZ could be safe for the aquatic organisms studied.

Conclusions

In the light of the results of this work, the following conclusions can be drawn:

(i) The advanced ozonation processes used achieved efficiencies of removal of carbamazepine higher than 99%, with levels of the drug at the treated solutions lower than the detection limit.
(ii) The treated solutions did not induce a marked acute toxicity to the bioindicators used.
(iii) The treated solutions (100%) induced the mortality of *D. magna* when occurred the increment of time exposition.
(iv) The diluted treated solutions (50 and 25%) from catalytic ozonation—TiO_2—of carbamazepine (200 μg/L) induced a decrease in the number of juveniles produced by female, the number of molts, and the body length.
(v) A solution of carbamazepine (200 μg/L) under catalytic ozonation—TiO_2, black light—diluted at 50 and 25% induced an increase in the number of juveniles produced by female and the body length.
(vi) The results emphasize the importance of carrying out not only acute but also chronic tests in aquatic organisms to assess more thoroughly the ecotoxicity of the resulting solutions after advanced ozonation processes, once chronic assays are more appropriate to detect the ecotoxicological impact of TPs formed during these treatments.

Once evaluated the CBZ toxicity to *D. magna* (Oropesa et al. 2016) and of solutions from selected chemical treatments applied to CBZ removal (this work) using Milli-Q water as matrix aqueous solutions, it could be interesting to evaluate (i) the TPs that occurred at the treated solutions and their acute and chronic toxicity to the same bioindicators used at the present study and (ii) the toxicity of domestic wastewaters spiked with this drug and also under the same chemical treatments. It is important to take into account that the domestic wastewaters are matrices of great complexity because they can be constituted by organic materials (detergents, pesticides, fat, oil and grease, coloring, solvents, phenols, and cyanide), nutrients (nitrogen, phosphorus, and ammonium), and inorganic materials (metals, acids, bases) (Henze and Comeau 2008) that might change the outcome of toxicity if compared to that found in Milli-Q water in this work. On the other hand, working with wastewaters is a more realistic scenario.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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